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Potential effect of earthworm *Eisenia fetida* extracts on the growth of *Fusarium oxysporum* f. sp. *cubense* tropical race- 4

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Abstract

Banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4), is a worrying destructive banana disease of which there is not yet effective control measures. The present study aimed to evaluate the ability of earthworms *Eisenia fetida* to be biological control agents against Foc TR4. Methodological approach consisted of assessing interactions between Foc TR4 and (i) enzyme β-N-Acetyl-glucosaminidase (NAGase) and (ii) *E. fetida* extracts that are the coelomic fluid (CF) and the crude crushed (CC). Then NAGase were dosed in *E. fetida* CF. Foc TR4 growth was inhibited by NAGase but no effect was observed with the extracts CF and CC of *E. fetida*. Enzymatic dosage showed that CF contained 0.015 ± 0.006IU/mg protein as NAGase activity. These results suggest the possible use of *E. fetida* in biocontrol of Foc TR4 however through a process other than the extracts CC and CF. The outcomes of this study may constitute background data allowing to explore potential of earthworms in biocontrol of banana pathogenic fungi, which is of great significance to the development of banana industry system and to the reduction in the use of fungicides.

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Introduction

Banana (*Musa* spp.) is one of the world's most important crops owing to its economic and food interest. Its fruits, namely cooking bananas (AAB, ABB, ...) and dessert bananas (AAA) are used in the diet of many populations in both importing and exporting countries (Lassoudière, 2007). In several tropical countries, plantain (AAB) is a staple food for various social strata. Plantain is an energetic food providing 120 kcal or 497 kJ per 100 g (Yao *et al.,* 2014). Its commercialization constitutes a source of income for rural or low-income populations (Ouina, 2017). Apart from bananas, other organs of banana plant such as pseudostem, leaves and peelings give rise to a wide variety of uses (animal feed, manufacture of industrial products) (Kumar *et al.,* 2012; Jyothirmayi and Rao, 2015).

Like any plant crop, banana plant is prone to attacks by bacteria, viruses, fungi, nematodes and weevils. Among these attacks, fungi have been for a long time a growing threat and lead to severe affections of the leaves, stems, fruits and roots, resulting in significant yield reductions (Stover, 1959; Viljoen, 2002; De Bellaire *et al.,* 2010; Dita *et al.,* 2018). *Fusarium* wilt is one of the most serious fungal disease that affect banana plant. It is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) and is responsible of constraints on banana production causing serious economic losses worldwide (Ploetz, 2015; Dita *et al.,* 2018. Based on the pathogenicity to host cultivars, Foc is divided into physiological races 1, 2 and 4. Unlike races 1 and 2 which affected respectively Gros Michel (AAA) and Manzano/Apple/Latundan (Silk, AAB), and cooking bananas of the Bluggoe (ABB) subgroup, race 4 has a broad host range. It infects almost all cultivars including "Dwarf Cavendish" (*Musa* sp. AAA group) as well as the hosts of race 1 and race 2 (Lassoudière, 2007; Sutherland *et al.,* 2013; Lin *et al.,* 2013). Race 4 has been split into subtropical race 4, which affects "Cavendish" and races 1 and 2 suscepts in the subtropics, and tropical race 4, which affects many of the same cultivars as subtropical race 4 in the tropics when disease-predisposing conditions are absent (Ploetz, 2015). Furthermore, vegetative compatibility which has been implemented owing to confusions of the race structure often happening in delineating strains of Foc, allow to identified a total of 24 vegetative compatibility groups (VCGs). Tropical race 4 is designated as VCG 01213/16 and subtropical race 4 belong to VCGs 0120, 0121, 0122, 0129 and 01211 (Dita *et al*., 2010; Mostert *et al*., 2017). *Fusarium* wilt, also known as Panama disease, affected several banana plantations in Australia, Taiwan, Philippines, India, Mozambique (Pegg *et al.,* 1996; Ploetz, 2015; Viljoen *et al.,* 2020) and South Africa (Viljoen, 2002). Control methods against *Fusarium* wilt that have been developed have focused on chemical (fungicide application) and cultural treatments, selection and varietal improvement by hybridization techniques (Bakry *et al.,* 2005; Lassoudière, 2007). However, these control methods have shown limitations in adapting or mutating pathogens, in inaccessibility of improved banana varieties to farmers with low incomes (Ploetz, 2005; Kra *et al.,* 2009).

Indeed, the banana cultivar "Gros Michel", which was the basis of banana export trade in Central America and resistant to *Fusarium* wilt, became sensitive in the years 1940 to 1950 and was replaced by the cultivar "Cavendish" (Ploetz, 2005). "Cavendish", the current export cultivar, has become sensitive since 1970 to Foc race 4 (Visser *et al.,* 2009). Fungicide use is increasingly criticized by consumer associations and scientists due to their harmful effects on environment and on human health are (Lassoudière, 2007; Cirad, 2011, Brühl and Zaller, 2019). Regarding worrying destructible effects of *Fusarium* wilt and galloping world demography (for example 48,796,000 inhabitants in 2050 in Côte d'Ivoire so the double of the current population) (UN, 2015), efforts to protect and develop the production of this staple

food should be intensified. Faced with the constraints related to the means of controlling *Fusarium* wilt previously mentioned, biological control is much explored as an alternative by the research (Gbongué *et al.,* 2012; Mohammed *et al.,* 2019; Torres-Trenas *et al.,* 2019).

Earthworms are soil invertebrates that participate in soil aeration and water infiltration, increasing the nutrients content of the soil, mixing soil minerals with organic material. All making these organisms soil fertility agents (Römbke *et al.,* 2005; Bhadauria and Saxena, 2010). In addition to this capacity of affecting positively soil functioning, earthworms were found to have potent antimicrobial activities. Indeed, they have developed innate immune mechanisms that detect pathogens by recognizing conserved molecular patterns (Prakash and Gunasekaran, 2011). Earthworm *Eudrilus eugeniae* paste showed inhibitory activity against pathogens such as bacteria *Staphylococcus aureus*, *Kebsiella pneumoniae* and *Salmonella abony*, and fungi *Candida albicans, Aspergillus flavus* and *Trichophytum rubrum* (Vasanthi *et al*., 2013).

According Pan *et al*. (2003), the coelomic fluid of the earthworm, *Eisenia fetida andrei* (Savigny) was demonstrated to possess an antimicrobial activity directed against earthworm pathogenic bacteria *Aeromonas hydrophila* and *Bacillus megaterium*. Thus, living in an environment with abundant pathogens, earthworms developed defense strategies against the living pathogens.

For instance, they have suspected to synthesize β-N-acetyl-glucosaminidase (NAGase), an enzyme that hydrolyses chitin, one of the main constituents ensuring the rigidity of fungal wall (Guthrie and Castle, 2006). These defense strategies or metabolite compounds allowing to implement defense strategies can be exploited for finding innovative biological solutions to issues related to above mentioned means of controlling *Fusarium* wilt.

This study proposes to evaluate the ability of earthworms to be biological control agents against the fungus Foc TR4. *Eisenia fetida* is a favorite worm species for composting and is frequently used as a biological monitor for experimental tests (OECD, 1984; Garg *et al.,* 2006; Ouina *et al.,* 2017). Specifically, interactions between Foc TR4 and (i) enzyme NAGase and (ii) *E. fetida* extracts (crude crushed and coelomic fluid) were assessed.

Materiel and methods

Identification of study materiel

The study material used in this work included earthworms *E. fetida* and the fungus *F. oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4). The earthworms were purchased from VersLaTerre (Pezanas, France). Foc TR4 was isolated from soil collected in some banana farms of the region of Lôh-Djiboua (Côte d'Ivoire), precisely in the localities of Divo (5°49'59 "N and 5°21'58" W) and Lakota (5°50'49 "N and 5°40'48" O).

Earthworm breeding

Earthworms purchased were breeded at the laboratory (University of Caen Normandie, France) with the help of a vermicomposting system (Worm Café) as described by Ouina *et al.* (2017). The vermicomposting was started with one kilogram of earthworms. At the composting set up, the vermicomposting system possessed two plates with one (work plate) containing earthworms and coconut fibre. The coconut fibre was used as bedding for earthworms which was obtained by fully soaking a coconut fibre block (from Ceylon Garden Coir) in six to seven litres of tepid water (38°C). A handful of kitchen wastes (vegetable products, eggshells and coffee grounds) and "Vita'Vers PLUS were put and mixed with earthworms and coconut fibre in the work plate. The whole was covered with humification mattress. The maintenance of the vermicomposting was then carried out by imputs of kitchen waste of approximately two kilograms

three times a week and then inputs of Vita'Vers PLUS (a handful) every week on the coconut fibre. Also, moistened paper towel was put every week in the work plate to provide earthworms with fibres. The vermicomposting system was kept at room temperature (18 ± 2°C). From the third month of composting, adult earthworms (weighing between 300 and 600mg) with well-developed clitella (OECD, 1984), were taken from the composting system for the subsequent analysis.

Isolation and preparation of pure culture of Foc TR4

Fungus Foc TR4 isolated in previous work (Ouina *et al.,* 2020) *via* culture dependant molecular approach, from rhizospheric soil samples collected in banana farms of the region of Lôh-Djiboua (Côte d'Ivoire) were cultivated on the culture media Potato Dextrose Agar (PDA). Petri dishes were incubated at 25°C for 7 days.

Research of anti-Foc TR4 activity of N-acetyl-glucosaminidase (NAGase)

N-acetyl-glucosamine, one of the constituents of the fungal wall, is the substrate for the enzyme NAGase. The test anti-Foc TR4 were performed using NAGase derived from a plant (*Canavalia ensiformis*) and NAGase of animal origin (bovine kidney) that respectively having specific activities of 23 UI /mg of protein and 10 to 50 UI /mg protein. For its implementation, the mycelium of a pure culture of Foc TR4 (obtained after seven days of incubation at 25°C on PDA) was mixed with 10mL of sterile distilled water and then crushed using a mixer (Moulinex, Caen, France). A quantity of 200µL of this Foc TR4 suspension was streaked by spreading on a Cristomalt agar. About two minutes after seeding, two sterile non-impregnated disks of diameter six millimetres each were deposited on the surface of the seeded agar. These disks were impregnated with 20µL of the enzyme NAGase the one and 20µL of sterile distilled water the other serving as a control (Bhattacharjee and Ghosh, 2015). Two tests were carried out for each of the NAGase enzymes. The different seeded Petri dishes were subsequently incubated at 25°C for 7 days.

Research of anti-Foc TR4 activity of crude crushed and cœlomic fluid of E. fetida

Preparation of crude crushed of E. fetida

Five mature earthworms according to the OECD (1984) criteria of the species *E. fetida*, were taken from the vermicomposting system, washed with tap water and then with sterile distilled water and dried using a paper towel. The earthworms were then crushed in an enameled porcelain hand mortar. The crushed obtained was immediately used for the inhibition tests.

Extraction of E. fetida's cœlomic fluid

The earthworm coelomic fluid was extracted according to the method reported by Bhattacharjee and Ghosh (2015). Twenty mature earthworms *E. fetida* were washed with tap water and then with sterile distilled water and dried with a paper towel. They were then placed in a sterile beaker and stimulated continuously for 15 min with 6 V DC generator. The stimulated worms ejected through their dorsal pores, the coelomic fluid. This liquid, collected in a two millilitres microtube, was centrifuged for 2 min at 13400g and the supernatant was used for inhibition tests.

Inhibition test of Foc TR4 growth

The inhibition tests of Foc TR4 growth by crude crushed and coelomic fluid of the earthworm *E. fetida* were performed as described for the assessment of the enzymes NAGase effect on Foc TR4 growth. In addition, concerning the test with earthworm crushed, the disc was impregnated with a portion of about 2mg of ground material.

Dosage of NAGase in E. fetida cœlomic fluid

The activity of NAGase in the coelomic liquid of earthworm *E. fetida*, was determined using the kit "test of β-N-acetyl glucosaminidase" *via* the method "test in the tubes". The implementation according to the manufacturer's instructions is as follows. A standard range was first carried out from the enzyme NAGase at dilution 10^{-2} (positive control). Different volumes (0; 0.5; 1;

2; 4 and 5µL) of NAGase suspension were diluted using NAGase dilution buffer to obtain final volumes of 5µL. In parallel, cœlomic fluid extracted from *E. fetida* (test sample) and diluted at 10^{-2} using the same dilution buffer used for NAGase, was distributed in different volumes (Table 1). The substrate solution (10mg of 4-Nitrophenyl N-acetyl-glucosaminide, 10mL of citrate buffer 0.09 mol. l^{-1}) beforehand incubated at 37 °C in a water bath for about 5 minutes, was added to each NAGase standard solution and each volume of coelomic fluid prepared. The reaction tubes (containing substrate and NAGase/coelomic fluid) (Table 1) were homogenized for 3 s and then incubated at 37 ° C for 30 min in the water bath. After incubation, the enzymatic reaction was stopped by adding 2mL of the stop solution (118mL of ultra-pure water, 5 g of Na_2 CO_3) to each tube. Each mixture, briefly homogenized, was transferred into 1mL spectrophotometer cuvettes. Absorbance of the mixtures was immediately measured at 405 nm using a spectrophotometer Spectronic Genesys 5 (Genesys, California, USA).

Table 1. Reagent composition of test sample and positive control tubes.

	Positive control									
NAGase standard solutions										
Specific activity (UI/mg of protein)	0	0.023	0.046	0.092	0.184					
Volumes (µL)	5	5	5	5	5					
Substrate solution (µL)	995	995	995	995	995					
Test sample										
Cœlomic fluid (µL)	10	20	30	40	50	60	70	80	90	100
Substrate solution (µL)	990	980	970	960	950	940	930	920	910	900
NAGase : N-acetyl glucosaminidase										

The "optical zero" of the spectrophotometer was made with distilled water. Three repetitions were carried out for these experiments (standard solution and coelomic fluid of earthworm). The NAGase activity of the coelomic liquid was determined through two steps: (i) Using the equation of trend curve "Optical density (OD) as a function of volume of *E. fetida* coelomic fluid", theoretical ODs were calculated using each of the different volumes of analyzed coelomic fluid. These theoretical ODs are the images of coelomic fluid volumes; (ii) Using the equation of the calibration curve of the pure enzyme NAGase of plant origin (*Canavalia ensiformis*), the volumes of pure NAGase or NAGase activities corresponding to the theoretical ODs previously determined, were calculated (Langlois and Rousset, 2015).

Statistical analyses

The data collected during the dosage of NAGase in *E. fetida* cœlomic fluid were entered using the Excel 2013 spreadsheet. The average and standard deviation values of ODs were determined using Statisca 7 software (StatSoft Inc, Tulsa, USA).

Results

Growth of Foc TR4 in presence of the enzyme NAGase and earthworm extracts

The enzymes N-acetyl-glucosaminidases (NAGase) of plant (*Canavalia ensiformis*, Jack bean) and animal (bovine kidney) origin, and earthworm *E. fetida* extracts (crushed crude and coelomic fluid) were assessed *in vitro* for their antagonist action on the growth of Foc TR4 (Fig. 1. and Fig. 2.). After incubation of the tested dishes for seven days, an inhibition zone of Foc TR4 mycelium was observed around the disks impregnated with the plant and animal NAGases enzymes, compared with the control disks. It is a circular zone with a diameter of about 40.5 mm and 54 mm, respectively for plant and animal NAGases. There were some spots of Foc TR4. Concerning *E. fetida* extracts, no inhibition zone of Foc TR4 mycelium was observed around the disks impregnated with both the crushed crude and the coelomic fluid.

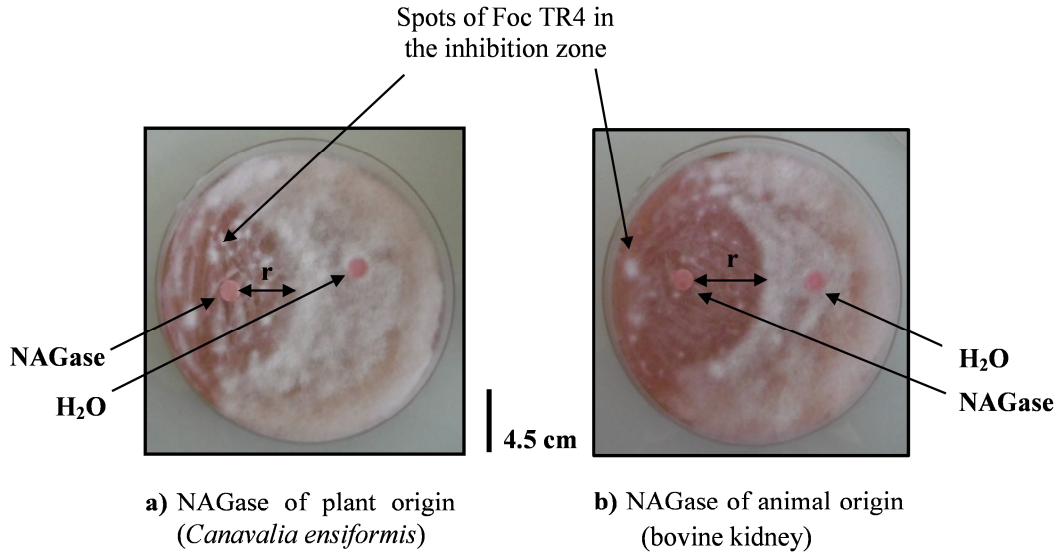

Fig. 1. Effect of N-Acetyl-glucosaminidase (NAGase) on the growth of *Fusarium oxysporum* f. sp. *cubense* race tropicale 4 (Foc TR4) (r: raduis of inhition zone).

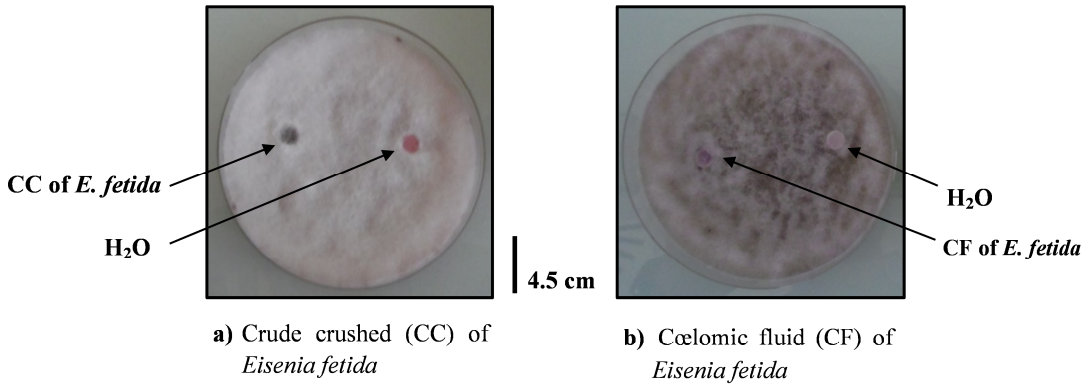

Fig. 2. Effect of crude crushed (CC) and cœlomic fluid (CF) of *Eisenia fetida* on the growth of *Fusarium oxysporum* f. sp. *cubense* race tropicale 4.

Activity of N-acetyl-glucosaminidase (NAGase) in the cœlomic fluid of *Eiseinia fetida*

Data about calibration of NAGase (*Canavalia ensiformis*), were observed in Fig. 3. After enzymatic reactions using standard solutions of NAGase, an increase of optical density at 405 nm was proportionally observed with the concentration of NAGase in each standard solution. The mathematical relationship modeling the calibration curve is y = 9.3565 x + 0.0157. The dosage of NAGase activity in different volumes of *E. fetida* coelomic fluid showed after enzymatic reaction with these volumes, an increase of OD with volume of tested coelomic fluid (Fig. 4.). The specific activity of the NAGase enzyme which is contained in each volume of analyzed coelomic fluid, is presented in the Table 2. After interpolation, only the volumes greater than or equal to 30µL of coelomic liquid at 10^{-2} of *E. fetida* allowed to obtain OD corresponding to suitable values of NAGase activity. Thus, based on the NAGase activity observed in different amounts (30 to 100µL) of 10^{-2} coelomic fluid, 1µL of coelomic fluid of earthworm *E. fetida* contains a NAGase activity which is equal to 0.015 ± 0.006 IU /mg protein.

Table 2. β-N-actyl-glucosaminidase (NAGase) activity of *Eisenia fetida* cœlomic fluid.

E. fetida cœlomic fluid at 10^{-2} (µL)	10	20	30	40	50	60	70	80	90	100
OD at 405 nm	-0.023	0.0027	0.0287	0.0547	0.0807	0.1067	0.1327	0.1587	0,1847	0,2107
NAGase activity in CF at 10^{-2} (UI/mg of protein)	-0.004	-0.001	0.001	0.004	0.007	0.01	0.0125	0.0152	0.0181	0.021

NAGase : β-N-acetyl-glucosaminidase ; OD : Optical Density

Negatives values of OD or NAGase activity indicate there is no NAGase activity in the corresponding volume of coelomic fluid

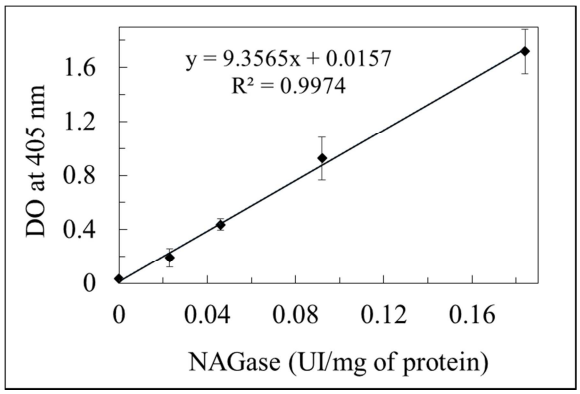

Fig. 3. Calibration curve of the enzyme β-N-Acetyl-glucosaminidase (NAGase) of plant origin (*Canavalia ensiformis*).

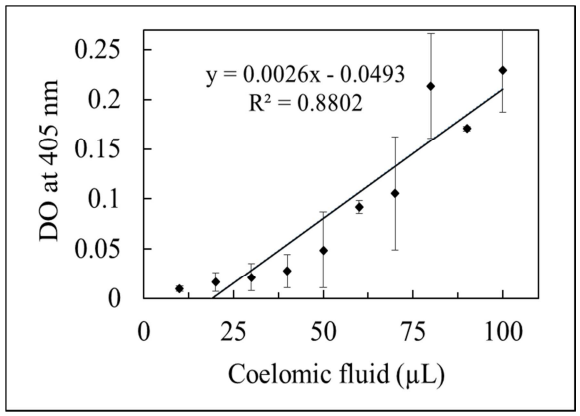

Fig. 4. Evolution of optical density as a function of volume of *E. fetida* cœlomic fluid.

Discussion

Fusarium oxysporum f. sp. *cubense* tropical race 4 (Foc TR4), is a pathogenic and ubiquitous soil fungus, responsible for banana *Fusarium* wilt, one of the most destructive fungal diseases for banana (*Musa* spp.). There is almost no resistance to Foc TR4 in all Cavendish type banana cultivars (Chen *et al*. 2019). Despite the loss of production and therefore the resulting food insecurity, control of Foc TR4 remains limited to prophylactic measures of which biological control and creation of resistant varieties are nowadays much explored by research. In order to contribute to the biological control against Foc TR4, the interactions between a model earthworm, *Eisenia fetida*, and Foc TR4 were evaluated in this study. Inhibition tests of Foc TR4 growth carried out *in vitro* with the enzymes NAGase, showed inhibition zones of Foc TR4 mycelium with 40.5mm and 54mm diameter respectively for the NAGase of plant and of animal origin. These inhibitions would be related to the presence of N-acetyl-glucosamine which is the NAGase substrate, among the components of Foc TR4 cell wall. Indeed, the cell wall of micromycetes is a supramolecular structure whose physical and chemical properties depend on its composition and the arrangement of its constituents. Chitin, one of the main constituents ensuring the rigidity of this wall, is a linear polymer in β (1-4) linkage of N-acetyl-glucosamine (Al-Askar *et al*., 2015; Min *et al*., 2020). This polysaccharide and many others are widely distributed in many micromycetes including *F. oxysporum* (Garcia-Rubio *et al*., 2020; Min *et al*., 2020).

Thus, in the presence of NAGase, the β (1-4) bonds between the different N-acetylglucosamine molecules are hydrolysed. This results in disruption of chitin and thus, fungal cell wall destruction and inhibition of Foc TR4 growth. Like Foc TR4 mycelium, inhibition of fungal growth in the presence of NAGases was observed in other fungi such as *Aspergillus niger, Penicillium* sp. and *Sclerotinia sclerotiorum* (Silva *et al.,* 2011; Li *et al*., 2021).

The difference observed in the inhibition diameters of the Foc TR4 mycelium could be due to the fact that the specific activities of the tested enzymes NAGase are different. Specific activity being an indicative parameter of the enzyme purity (Nelson and Cox, 2013), these data suggest that the NAGase of animal origin has a greater specificity for Foc TR4 and a high degree of purity compared to the NAGase of plant origin. The presence of Foc TR4 spots in the inhibition zones would indicate the resistant character of these spots to the enzymes NAGase. It could also suggest that NAGases in contact with Foc TR4 and incubated for 7 days would have inhibited Foc TR4 mycelium for a certain period of time, but they were later denatured. This again resulted in Foc TR4 growth in the inhibition zone.

Inhibition of Foc TR4 mycelium in the presence of the enzymes NAGase, shows that these enzymes have antifungal activity directed against this phytopathogenic micromycete. Thus, some or all organisms that produce NAGase may also perform this anti-Foc TR4 activity. The verification of this hypothesis was carried out through the interaction tests between Foc TR4 and the extracts (crude crushed and coelomic fluid) of the earthworm *E. fetida*. In presence of these extracts, no inhibition of Foc TR4 mycelium was observed. This could be explained on the one hand by the absence of the enzyme NAGase in products extracted from *E. fetida*.

On the other hand, the NAGase possibly present in the extracts of *E. fetida* could be inhibited by the organic matter contained in these extracts. Indeed, the organic matter is a complex set including, among others, lipid, carbohydrates, proteins compounds and nucleic acids that interact with each other. These different interactions manifest themselves for example in the blockage of the active sites of enzymes. This can lead to inhibition of their enzymatic activities (Berg *et al.,* 2002; Błońska *et al*., 2017). Kalembasa and Symanowicz (2012) report that the supply of organic matter in a clay-loam soil resulted in a significant decrease in urease activity in this soil. The enzyme NAGase, possibly present in *E. fetida* extracts, could be in low concentration to cause Foc TR4 inhibition. Ansari and Sitaram (2011) reported growth inhibition of the fungi *Candida albicans* and *Aspergillus flavus* by extracts of *E. fetida*. In view of their work, the absence of Foc TR4 inhibition by the extracts of this worm could suggest that it does not exhibit in its extracts, an inhibitory activity against all micromycetes.

The responses of Foc TR4, which are inhibition of its mycelium in presence to enzymes NAGase and absence of inhibition in the presence of *E. fetida* extracts, led to the search for a NAGase activity in the coelomic fluid of *E. fetida*. Calibration of the NAGase of plant origin gave a curve whose mathematical characteristics are $y = 9.3565 x + 0.0157$ and $R^2 = 0.99$. The characteristic $R^2 = 0.99$ indicates that the optical density (OD) obtained after enzymatic reaction from the standard solutions are proportional to the NAGase concentrations of these standard solutions (Mendham *et al*., 2005; Nakagawa *et al.*, 2017). The evolution of OD with the coelomic fluid volume of *E. fetida* indicates that the activity of NAGase increases with the volume of coelomic fluid. This enzymatic assay shows that 1µL of coelomic fluid extracted from *E. fetida*, contains NAGase activity which is equal to 0.015 ± 0.006 IU /mg of protein. This result is in agreement with that of the work of Honsi and Stenersen (2000), indicating that the coelomic fluid of *E. fetida*, contains a NAGase activity of value 0.012 µmol / min /mg protein. The presence of a NAGase activity in the coelomic fluid of *E. fetida* suggests, with regard to the composition of the cell wall of *F. oxysporum* (Min *et al*., 2020), that this earthworm can be used to control Foc TR4. However, inhibition of Foc TR4 not observed in the presence of coelomic fluid indicates that the NAGase activity dosed in this earthworm extract would be lower than the MIC of the enzyme NAGase regarding Foc TR4.

Conclusion

This study being included in the framework of the biological control against Foc TR4, allowed to assess the interactions between a model earthworm, *Eisenia fetida*, and Foc TR4. The pathogenic agent of banana *Fusarium* wilt (Foc TR4) developed well in the presence of the extracts (crude crushed and coelomic fluid) of the earthworm *E. fetida*. But it is sensitive to the commercial enzymes NAGase of plant (*Canavalia ensiformis*) and animal (cattle kidney) origin. The coelomic fluid of *E. fetida* contains a NAGase activity that is weak to inhibit Foc TR4 growth. The outcomes of this work may constitute background data allowing to explore potential of earthworms in biocontrol of banana pathogenic fungi, which is of great significance to the development of banana industry system and to the reduction in the use of fungicides.

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Conflict of interest

The authors have declared that no competing interest exists.

References

Al-Askar AA, Baka ZA, Rashad YM, Ghoneem KM, Abdulkhair WM, Hafez EE, Shabana YM. 2015. Evaluation of *Streptomyces griseorubens* E44G for the biocontrol of *Fusarium oxysporum* f. sp. *lycopersici*: ultrastructural and cytochemical investigations. Annals of Microbiology **65,** 1815-1824.

Ansari AA, Sitaram K. 2011. An investigation into the anti-microbial and anti-fungal properties of earthworm powder obtained from *Eisenia fetida.* American Journal of Food Technology **6,** 329-335.

Bakry F, Carreel F, Horry JP, Jenny C, Tomekpe K. 2005. La diversité génétique des bananiers cultivés: situation actuelle et perspectives. "Le Sélectionneur Français" **55,** 3341.

Berg JM, Tymoczko JL, Stryer L. 2002. Biochemistry, 5th edition. New York, USA: W H Freeman and Company 1515 p.

Bhadauria T, Saxena KG. 2010. Role of earthworms in soil fertility maintenance through the production of biogenic structures. Applied and Environmental Soil Science **2010,** 7 p. https://doi.org/10.1155/2010/816073.

Bhattacharjee G, Ghosh AC. 2015. Study of antibacterial properties in coelomic fluid from the earthworm *Eutyphoeusgammiei* (Beddard). International Journal of Biological & Pharmaceutical Research **6,** 73-77.

Błońska E, Lasota J, Gruba P. 2017. Enzymatic activity and stabilization of organic matter in soil with different detritus inputs. Soil Science and Plant Nutrition **63,** 242-247

Brühl CA, Zaller JG. 2019. Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Frontiers in Environmental Science **7,** 4 p. https://doi.org/10.3389/fenvs.2019.00177.

Chen A, Sun J, Matthews A, Armas-Egas L, Chen N, Hamill S, Mintoff S, Tran-Nguyen LTT, Batley J, Aitken EAB. 2019. Assessing variations in host resistance to *Fusarium oxysporum* f sp. *cubense* race 4 in *Musa* species, with a focus on the subtropical race 4. Frontiers in Microbiology **10,** 13 p.

Cirad. 2011. Chlordecone in Antilles. https://antilles-guyane.cirad.fr/publications-ressources/dossiers-thematiques/chlordecone (accessed 20 june 2018).

De Bellaire LL, Fouré E, Abadie C, Carlier J. 2010. Black Leaf Streak Disease is challenging the banana industry. Fruits **65,** 327-342.

Dita M, Barquero M, Heck D, Mizubuti ESG, Staver CP. 2018. *Fusarium* wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. Frontiers in Plant Science **9**, 1468.
DOI: 10.3389/fpls.2018.01468.

Dita MA, Waalwijk C, Buddenhagen IW, Souza JMT, Kema GHJ. 2010. A molecular diagnostic for tropical race 4 of the banana *Fusarium* wilt pathogen. Plant Pathology **59,** 348-357.

Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N. 2020. The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* species. Frontiers in Microbiology **10,** 13 p https://doi.org/10.3389/fmicb.2019.02993.

Garg P, Gupta A, Satya S. 2006. Vermicomposting of different types of waste using *Eisenia foetida*: A comparative study. Bioresource Technology **97,** 391-395.

Gbongué LR, Diabaté S, Bomisso EL, Dick E, de Franqueville H, Koné D. 2012. Étude de la confrontation des souches pathogènes et non pathogènes de *Fusarium oxysporum* dans l'acquisition de la résistance contre la fusariose du palmier à huile. Journal of Animal and Plant Sciences **15,** 2171-2183.

Guthrie JL, Castle AJ. 2006. Chitinase production during interaction of *Trichoderma aggressivum* and *Agaricus bisporus*. Canadian Journal of Microbiology **52,** 961-967.

Honsi TG, Stenersen J. 2000. Activity and localisation of the lysosomal marker enzymes acid phosphatase, N-acetyl-b-D-glucosaminidase, and b-galactosidase in the earthworms *Eisenia fetida* and *E. veneta*. Comparative Biochemistry and Physiology Part B **125,** 429-437.

Jyothirmayi N, Rao NM. 2015. Banana Medicinal Uses. Journal of Medical Science and Technology **4,** 152-160.

Kalembasa S, Symanowicz B. 2012. Enzymatic activity of soil after applying various waste organics materials, ash and mineral fertilizers. Polish Journal of Environmental Studies **21,** 1635-1641.

Kra KD, Diallo HA, Kouadio YJ. 2009. Activités antifongiques de l'extrait de *Chromolaena odorata* (L.) King & Robins sur deux isolats de *Fusarium oxysporum* (E.F. Sm.) responsables du jaunissement mortel des feuilles des bananiers. Journal of Applied Biosciences **24,** 1488-1496.

Kumar KS, Bhowmik D, Duraivel S, Umadevi M. 2012. Traditional and medicinal uses of banana. Journal of Pharmacognosy and Phytochemistry **1,** 51-63.

Langlois E, Rousset D. 2015. Calibration methods for the quantification of pollutants. http://www.inrs.fr/dms/inrs/PDF/metropol-analyse-etalonnage.pdf (accessed 20 june 2016).

Lassoudière A. 2007. Banana tree and its culture, *Quæ*, Paris, France 383 p.

Li T, Li L, Du F, Sun L, Shi J, Long M, Chen Z. 2021. Activity and mechanism of action of antifungal peptides from microorganisms: a review. Molecules **26,** 18 p.

Lin YH, Su CC, Chao CP, Chen CY, Chang CJ, Huang JW, Chang PFL. 2013. A molecular diagnosis method using real-time PCR for quantification and detection of *Fusarium oxysporum* f. sp. *cubense* race 4. European Journal of Plant Pathology **135,** 395-405.

Mendham J, Denney RC, Barnes JD, Thomas MJK. 2005. Vogel's textbook of quantitative chemical analysis 6th edition, De Boeck Supérieur, Belgique 836 p.

Min K, Naseem S, Konopka JB. 2020. N-Acetylglucosamine regulates morphogenesis and virulence pathways in fungi. Journal of Fungi **6,** 16 p.

Mohammed BL, Hussein RA, Toama FN. 2019. Biological control of *Fusarium* wilt in tomato by endophytic rhizobactria. Energy Procedia **157,** 171-179.

Mostert D, Molina AB, Daniells J, Fourie G, Hermanto C, Chao C-P, Fabregar E, Sinohin VG, Masdek N, Thangavelu R, Li C, Yi G, Mostert L, Viljoen A. 2017. The distribution and host range of the banana *Fusarium* wilt fungus, *Fusarium oxysporum* f. sp. *cubense*, in Asia. PLoS ONE **12(7),** e0181630. https://doi.org/10.1371 /journal.pone.0181630.

Nakagawa S, Johnson PCD, Schielzeth H. 2017 The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed effects models revisited and expanded. Journal of the Royal Society Interface **14,** 11 p.

Nelson DL, Cox DM. 2013. Principles of Biochemistry, England, UK: Macmillan 1158 p.

OECD. 1984. OECD Guideline for Testing of Chemicals, No. **207,** Earthworm Acute Toxicity. Organisation for Economic Co-operation and Development: Paris.

Ouina TS, Panoff JM, Gente S, Garon D, Rioult JP, Gonnety TJ, Koussémon-Camara M. 2020. *Cropping practices and fungal contamination* in banana plantations in Côte d'Ivoire. Research Journal of Microbiology **15,** 98-108.

Ouina TS, Panoff JM, Koussémon M, Gonnety TJ. 2017. Effect of pesticides and micro-organisms on earthworm *Eisenia fetida* (Savigny, 1826). African Journal of Agricultural Research **12,** 705-715.

Ouina TS. 2017. Suitability of the earthworm *Eisenia fetida* for biological control of *Fusarium oxysporum* f. sp. *cubense* tropical race 4, a pathogen of *Fusarium* wilt of banana. PhD thesis, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire 187 p.

Pan W, Liu X, Ge F, Zheng T. 2003. Reconfirmation of antimicrobial activity in the coelomic fluid of the earthworm *Eisenia fetida andrei* by colorimetric assay. Journal of Biosciences **28,** 723-731.

Pegg KG, Moore NY, Bentley S. 1996. *Fusarium* wilt of banana in Australia: a review. Australian Journal of Agricultural Research **47,** 637-650.

Ploetz RC. 2005. Panama disease: an old nemesis rears its ugly head, part 1: the beginnings of the banana export trades. Plant Health Progress **7,** 22 p. Doi: 10.1094.

Ploetz RC. 2015. *Fusarium* wilt of Banana. Phytopathology **105,** 1512-1521.

Prakash M, Gunasekaran G. 2011. Antibacterial activity of the indigenous earthworms *Lampito mauritii* (Kinberg) and *Perionyx excavatus* (Perrier). The Journal of Alternative and Complementary Medicine **17,** 167-170.

Römbke J, Jänscha S, Diddenb W. 2005. The use of earthworms in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety **62,** 249-265.

Silva DM, Batista LR, Rezende EF, Fungaro MHP, Sartori D, Alves E. 2011. Identification of fungi of the genus *Aspergillus* section nigri using polyphasic taxonomy. Brazilian Journal of Microbiology **42,** 761-773.

Stover RH. 1959. A rapid and simple pathogenicity test for detecting virulent clones of *Fusarium oxysporum* f. sp. *cubense* using seedlings of *Musa balbisiana.* Nature **184,** 1591-1592.

Sutherland R, Viljoen A, Myburg AA, van den Berg N. 2013. Pathogenicity associated genes in *Fusarium oxysporum* f. sp. *cubense* race 4. South African Journal of Science **109,** 1-10.

Torres-Trenas A, Prieto P, Cañizares MC, García-Pedrajas MD, Pérez-Artés E. 2019. Mycovirus *Fusarium oxysporum* f. sp. *dianthi* Virus 1 decreases the colonizing efficiency of its fungal host. Frontiers in Cellular and Infection Microbiology **9,** 12 p. https://doi.org/10.3389/ fcimb .2019.00051

UN (United Nations). 2015. Department of Economic and Social Affairs, Population Division (2015) World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241. 66 p.

Vasanthi K, Chairman K, Singh AJAR. 2013. Antimicrobial activity of earthworm (*Eudrilus eugeniae*) paste. African Journal of Environmental Science and Technology **7,** 789-793. DOI: 10.5897/AJEST2013.1455.

Viljoen A, Mostert D, Chiconela T, Beukes I, Fraser C, Dwyer J, Murray H, Jamisse A, Matabuana EL, Tazan G, Amugoli OM, Mondjana A, Vaz A, Pretorius A, Bothma S, Rose LJ, Beed F, Dusunceli F, Chao CP, Molina A. 2020. Occurrence and spread of the banana fungus *Fusarium oxysporum* f. sp. *cubense* TR4 in Mozambique. South African Journal of Science **116,** 11 p. https://doi. org/10.17159/sajs.2020/8608.

Viljoen A. 2002. The status of *Fusarium* wilt (Panama disease) of banana in South Africa. South African Journal of Science **98,** 341-344.

Visser M, Gordon T, Fourie G, Viljoen A. 2009. Characterization of South African isolates of *Fusarium oxysporum* f. sp. *cubense* from Cavendish bananas. South African Journal of Science **106,** 6 p.

Yao AK, Koffi DM, Irié ZB, Niamke SL. 2014. Conservation of plantain banana (Musa AAB) in the green state by using polyethylene films of different thicknesses. Journal of Animal and Plant Sciences **23,** 3677-3690.